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The Internal Meristem Layer (L3) Determines Floral Meristem Size and Carpel Number in Tomato Periclinal Chimeras

Eugene J. Szymkowiak[1] and Ian M. Sussex[2]

Department of Biology, Osborn Memorial Laboratories, Yale University, New Haven, Connecticut 06511

Cell–cell interactions are important during plant development. We have generated periclinal chimeras between plants that differ in the number of carpels per flower to determine the roles of cells occupying specific positions in the floral meristem in determining the number of carpels initiated. Intraspecific chimeras were generated between tomato (*Lycopersicon esculentum*) expressing the mutation *fasciated*, which causes an increased number of floral organs per whorl, and tomato wild type for *fasciated*. Interspecific chimeras were generated between tomato and *L. peruvianum*, which differ in number of carpels per flower. In both sets of chimeras, carpel number as well as the size of the floral meristem during carpel initiation were not determined by the genotype of cells in the outer two layers of the meristem (L1 and L2) but were determined by the genotype of cells occupying the inner layer (L3) of the meristem. We concluded from these experiments that during floral organ initiation, cells in certain layers of the meristem respond to information supplied to them from other cells in the meristem.

INTRODUCTION

Throughout postembryonic development in higher plants, organ primordia are initiated at predictable sites on shoot apical meristems. Organ primordia are derived from many cells (Poethig and Sussex, 1985) that originate from several cell lineages in the meristem (Tilney-Bassett, 1986). Thus, during organ initiation, a large group of cells from multiple lineages must receive a signal to commence initiation and then coordinately change their growth pattern and form a determinate appendage. The nature of the information that coordinates cell function during organ primordium initiation and the means by which it is communicated among cells are unknown.

In dicotyledonous plants, three separate layers of cells are usually present in a shoot meristem. The three layers (L1, L2, and L3) result from the restriction of cell division in the outer two layers to an anticlinal plane, whereas cells underlying these layers divide in any plane. Periclinal polyploid cytochimeras of Datura were first used to demonstrate the existence of three distinct cell lineages in the shoot meristem (Satina et al., 1940). Examination of leaves, sepals, petals, stamens, and carpels of the cytochimeras showed that cells derived from all three meristem layers participated in the formation of the primordia of all of these organs (Satina and Blakeslee, 1941, 1943).

Although all three meristem layers participate in organogenesis, the contribution of cells derived from the three layers to mature vegetative and floral organs is variable, and yet organs of regular morphology are produced (Stewart et al., 1974; Stewart and Dermen, 1975). Furthermore, cells from one layer are capable of differentiating as cells usually derived from another layer if they become incorporated into this layer as a result of atypical periclinal divisions (Stewart and Burk, 1970; Stewart and Dermen, 1973). Therefore, during plant development the pattern of cell division and differentiation must depend on positional information and cellular interactions. Mosaic plants containing x-ray–induced genetically marked clonal sectors have been useful in investigating cellular interactions during development. For example, in maize plants mosaic for cells carrying the *Knotted* mutation, the division patterns of epidermal cells of the leaf involved in knot formation depend on the genotype of middle mesophyll cells and not on their own genotype (Hake and Freeling, 1986; Sinha and Hake, 1990).

Genetically mosaic plants such as those examined in the analysis of *Knotted* are generated by a change in the genotype of a cell during the plant's development. This approach is limited by the chromosomal arrangement of the gene of interest and appropriate cell layer marker genes. However, chimeras can also be generated by the incorporation of cells from two or more different plants into a single meristem, potentially forming a stable periclinal chimera (Marcotrigiano and Gouin, 1984; Tilney-Bassett, 1986; Binding et al., 1987). Chimeras generated in this manner allow the analysis of developmental interactions between cells that differ for a single gene, regardless of chromosomal location, or between cells that differ for multiple genetic traits, or between cells from

[1] To whom correspondence should be addressed at Department of Biological Sciences, University of Iowa, Iowa City, IA 52242.

[2] Current address: Department of Plant Biology, University of California, Berkeley, CA 94720.

Figure 1. Examples of Cell Layer Markers in Sectored Shoots and Periclinal Chimeras.

different species or genera. One such graft-generated periclinal chimera, Camellia + "Daisy Eagleson," provides information concerning the regulation of the differentiation of floral organs. This chimera had L1 of *Camellia sasanqua* and L2 and L3 of *C. japonica*. In the chimera, L1-derived, *C. sasanqua* cells appeared to dictate the pattern of differentiation of *C. japonica* internal cells during the development of floral organs (Stewart et al., 1972). Although a chimera with the reciprocal cell layer arrangement was not obtained, the development of this chimera suggests that cells in L1 of the chimera provide information critical for determining the pattern of organ differentiation. Cells occupying L1 of the meristem have also been implicated in determining the pattern of organ initiation through the arrangement of cellulose microfibrils in their outermost wall (Green, 1988).

We are interested in the processes that coordinate the activities of cells in the apical meristem during organ initiation. We have generated a series of periclinal chimeras between plants having different numbers of carpels to investigate how cells located in the three meristem layers are coordinated during organ initiation. In one set of intraspecific chimeras, the source of variation in carpel number was the mutation *fasciated*, which causes an increased number of carpels in tomato flowers (MacArthur, 1928). A second set of interspecific chimeras was generated between *Lycopersicon peruvianum*, which forms two carpels per flower, and a line of tomato (*L. esculentum*) that forms more than two carpels per flower. In these chimeras, L1 and L2 cells participated in carpel initiation by responding to information provided by underlying L3 cells. The genotype of cells in L3 or their derivatives was the major determinant both of meristem size during carpel initiation and of the number of carpels initiated. Therefore, during carpel initiation, cells in specific locations in the meristem (L3) coordinated the development of the other cells.

RESULTS

Cell Layer Identity of Chimeras

The chimeras were identified by novel combinations of the genetic markers carried by the plants grafted together to generate the chimeras. Two chimeras were obtained from a graft having a scion of tomato homozygous for cell layer markers *hairless* (*h*), *Xanthophyllic-2* (*Xa-2*), and *anthocyanin gainer* (*ag*), and that was wild type for *fasciated*. This plant will be referred to as +++ because the cells in all three meristem layers (L1, L2, and L3) were wild type for *fasciated*. The stock used in the graft was a tomato plant wild type for the three marker mutations and homozygous for *fasciated* (fff). A shoot having three major sectors of different cell layer combinations developed from the surface of the graft junction that was exposed after most of the scion was cut away, as shown in Figure 1A. One sector on this shoot was yellow, indicating the presence of *Xa-2* cells, lacked anthocyanin, indicating the presence of *ag* cells, and had *hairless* trichomes, indicating that the cell layer arrangement of this sector was +++. The second sector was green with wild-type trichomes and anthocyanin, indicating that the cell layer arrangement of this sector was fff. The third sector had epidermal trichomes characteristic of *hairless* carried by the +++ scion and green color and normal anthocyanin expression of the fff stock, indicating that the cell layer arrangement was +ff. A bud that developed in a leaf axil entirely contained within the +ff sector developed as the periclinal chimera +ff. A shoot that developed from a node at the border of the fff and +++ sectors eventually yielded a periclinal chimera having the cell layer arrangement ++f. The identity of the ++f chimera was especially apparent in its leaves, which were variegated with yellow margins (L2-derived) and green centers (L3-derived) and had *hairless* epidermal trichomes (L1-derived). This pattern was also apparent in the sepals of the flowers.

The genotype of L2 in the chimeras was further verified by self-pollinating each chimera and examining the phenotypes of the progeny, because gametes are generally formed from L2-derived cells (Tilney-Bassett, 1986). All 25 progeny of self-pollinated +ff were identical to the fff graft partner, confirming the identity of L2 cells as *fasciated*, and all 25 progeny of self-pollinated ++f were identical to the +++ graft partner, confirming the identity of L2 cells as wild type. Small yellow sectors on +ff leaves have been observed very infrequently, indicating a late periclinal division resulting in an L1-to-L2 replacement event. These sectors provided additional evidence that L1 of chimera +ff is wild type. Several +++ sectors developed on chimera ++f and eventually grew as +++ shoots. These shoots were identical in phenotype to the +++ graft partner. Shoots regenerated from the cut stem surfaces of rooted shoots of ++f have yielded both +++ and fff type plants, identical in phenotype to the original graft partners.

Figure 1. (continued).

(A) Sectored shoot growing from cut through graft junction between *fasciated* tomato and tomato wild type for *fasciated* but expressing cell layer marker mutations.
(B) Sectored shoot growing from cut through graft junction between tomato expressing cell layer marker mutations and *L. peruvianum*.
(C) Chimera PrEPr.
(D) Chimera PrPrE.
(E) Chimera PrPrE fruit (red tissue, L3-derived tomato; white tissue, L2-derived *L. peruvianum*).
(F) Chimera PrEE leaf with green sector as a result of L1 to L2 periclinal division.
Arrows indicate the positions of graft junctions.

These shoots are useful in demonstrating that in chimera ++f, both wild-type and *fasciated* cells were present in the chimera.

Chimeras between tomato and *L. peruvianum* were obtained from a graft that had a scion of *L. peruvianum* (PrPrPr) and a stock of tomato (*L. esculentum*, EEE) carrying the marker mutations *Xa-2* and *Lanata* (*Ln*). A shoot, shown in Figure 1B, developed with three distinct sectors. The majority of the shoot was green with *L. peruvianum* epidermal trichomes, indicating that in this sector *L. peruvianum* cells were in all three meristem layers, PrPrPr. A second small sector had tomato cells in all three meristem layers, EEE, as indicated by *Lanata* epidermal trichomes and the yellow, *Xa-2* L2- and L3-derived cells. A scanning electron micrograph of the upper leaf surface at the border of these two sectors is shown in Figure 2. A third sector consisted of the cell layer combination PrEE. An axillary bud in the PrEE sector developed as a periclinal chimera that had *L. peruvianum* trichomes, indicating that L1 was composed of *L. peruvianum* cells, and yellow leaves and stem, indicating that L2 and L3 were composed of tomato cells.

Shoot regeneration from cultured explants of chimeras has been proven to be useful in generating nonchimeric shoots and chimeric shoots with cell layer arrangement different from the original chimera (Marcotrigiano, 1986). Leaf discs of chimera PrEE were placed on shoot-inducing medium. Many EEE and PrPrPr shoots were obtained, confirming that both *L. peruvianum* and tomato cells are present in chimera PrEE. One regenerated shoot consisted of a sector containing *L. peruvianum* in all three cell layers (PrPrPr) and a second sector with the cell layer combination of the original leaf disc (PrEE). From this shoot, two different sectors later developed. One sector had the cell layer combination PrEPr and eventually yielded a periclinal chimera of the same cell layer arrangement. This chimera (PrEPr), shown in Figure 1C, had *L. peruvianum* trichomes and leaves with yellow margins and green centers, indicating that L2 was composed of tomato cells and that L3 was composed of green *L. peruvianum* cells. The second sector had the cell layer arrangement PrPrE and yielded the periclinal chimera PrPrE shown in Figure 1D. This chimera had *L. peruvianum* epidermal trichomes and leaves with yellow-green centers and darker green margins. This pattern was particularly obvious in young, expanding leaves and indicated that L2 was composed of green *L. peruvianum* cells and that L3 was composed of yellow tomato cells.

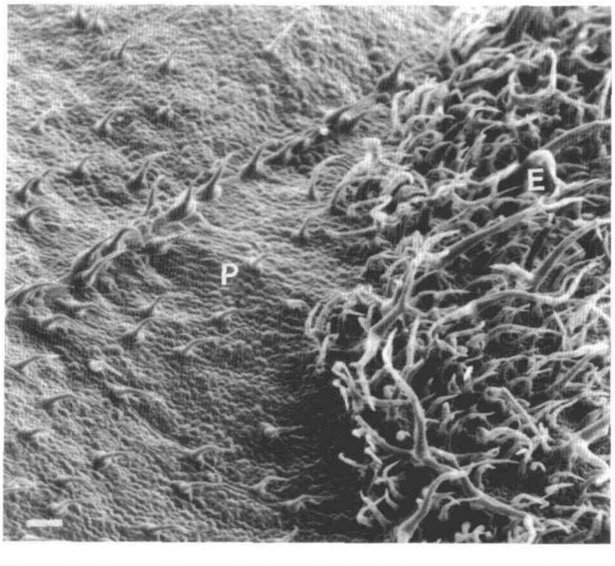

Figure 2. Sectored (PrPrPr/EEE) Adaxial Leaf Surface from the Sectored Shoot Shown in Figure 1B.

E indicates EEE-derived tissue. P indicates PrPrPr-derived tissue. Bar = 100 μm.

Although all of the tomato–*L. peruvianum* chimeras had flowered and set fruit, none had produced seeds. Thus, it was not possible to use self-pollinated progeny to confirm the identity of L2 cells in these chimeras. This may be due to the expression of the *S* locus incompatibility genes in the *L. peruvianum* cells comprising L1 of all three of the chimeras. Alternatively, it may be due to other incompatible interactions between the two species that interfere with reproduction. However, because *L. peruvianum* fruit are green and tomato fruit are red, the contribution of cells from the different layers of the chimera is apparent, as shown in Figure 1E.

Differences in the normal contribution of the derivatives of cells in each meristem layer to the mature organs of the chimeras, due to occasional cell divisions in atypical planes, have been observed. These events were useful in confirming the cell layer identities of the chimeras. For example, in chimera PrEE, L1-to-L2 replacement events as a result of periclinal divisions in L1 have been observed as small green sectors on leaves (Figure 1F). L2-to-L1 displacement events resulting from periclinal divisions in L2 have also been observed in the same chimera. These sectors are especially obvious because the tomato cells in the chimera carry the *Lanata* mutation, which is expressed in epidermal cells. On one occasion, an axillary bud developed within such a sector and eventually produced a pure tomato shoot. This EEE shoot had a phenotype identical to the original tomato graft partner. EEE shoots have also been regenerated from decapitated, rooted cuttings of PrEE.

Both *L. peruvianum* (PrPrPr) and tomato (EEE) shoots identical in phenotype to the original graft partners have been obtained from chimera PrEPr. This chimera often develops PrEE sectors that yield PrEE periclinal chimeras. These PrEE shoots are identical in phenotype to the original PrEE chimera.

Although the sectors described above were not commonly observed, many PrPrPr sectors developed on chimera PrPrE. These sectors yielded pure *L. peruvianum* shoots. A single shoot of EEE developed from an axillary bud of chimera PrPrE. Both the PrPrPr shoots and the EEE shoot were identical in phenotype to the plants used to generate the chimera.

Determination of Floral Organ Number

During flower development in tomato, the meristem initiates primordia in an acropetal sequence of sepals, petals, stamens, and carpels. The sepals are initiated successively in a spiral

Table 1. Floral Organ Number of Wild Type (+ + +), *fasciated* (fff), and Chimeras +ff and + +f[a]

	Sepal	Petal	Stamen	Carpel	*n*
+ + +	5.7 (0.9[b]) x	5.8 (0.9) x	6.1 (1.1) x	4.0 (1.4) x	33
fff	8.1 (1.6) y	10.5 (3.0) y	14.2 (4.4) y	17.4 (5.0) y	21
+ff	8.0 (1.5) y	9.7 (2.6) y	13.7 (5.0) y	16.2 (4.2) y	32
+ +f	7.3 (1.4) y	8.2 (2.0) z	9.6 (3.3) z	11.9 (4.0) z	30

[a] Means for each organ type followed by a different letter (x, y, or z) differ significantly ($P < 0.0001$). Distances between means for each organ type were determined with the Student-Newman-Keuls test.
[b] SD.

sequence, whereas petals, stamens, and carpels appear to be initiated in whorls. Organs in each successive whorl are positioned alternate to organs in the previous whorl (Sawhney and Greyson, 1972; Sekhar and Sawhney, 1984). Generally, five to six sepals, petals, and stamens, and two or more carpels are initiated, depending on the particular tomato line. In plants homozygous for *fasciated* (*f*), there is a great increase in the number of carpel primordia initiated by floral meristems (MacArthur, 1928).

Two chimeras were obtained between non-*fasciated* (wild type) tomato plants and tomato plants homozygous for *fasciated.* Mean values for the number of floral organs in each whorl for *fasciated* plants (fff), non-*fasciated* plants (+++), and chimeras +ff and ++f are given in Table 1. *fasciated* plants (fff) exhibited a progressive increase in the number of organs initiated in each whorl as compared to non-*fasciated* plants (+++). The number of organs per whorl of chimera +ff are identical to those of the *fasciated* plants, and organ numbers per whorl of chimera ++f are slightly less than those of the *fasciated* plants. Occasionally, as a result of atypical cell divisions in the L2 meristem layer, +++ shoots developed on chimera ++f. The number of floral organs on these shoots were identical to that of seed-derived, +++ plants. For example, the first four flowers to develop on one such shoot had three, four, four, and three carpels, respectively. This inflorescence developed on the shoot after three successive nonsectored +++ leaves were formed. Grafts of chimeras ++f and +ff onto stocks of +++ or fff plants showed no effect on the numbers of floral organs initiated by the scions. Similarly, the number of floral organs initiated by +++ scions were not influenced by fff stocks and vice versa (data not shown).

L. peruvianum has a pattern of floral organ initiation similar to that of tomato. The line of *L. peruvianum* and the line of tomato used to generate the chimeras did not differ significantly in number of sepals, petals, or stamens; therefore, only information concerning carpel number is reported. Three chimeras, PrEE, PrEPr, and PrPrE, were obtained between *L. peruvianum* and tomato. The number of carpels in mature flowers of *L. peruvianum*, tomato, and the three chimeras are reported in Table 2. Chimera PrEPr and *L. peruvianum* (PrPrPr) always formed two carpels, whereas tomato (EEE), PrEE, and PrPrE always formed more than two carpels. Flowers on PrEE shoots that developed as a result of atypical cell divisions in the L2 meristem layer on chimera PrEPr had the same number of carpels per flower as rooted PrEE plants. Similarly, PrPrPr shoots that developed as a result of atypical cell divisions in the L2 meristem layer on chimera PrPrE had two carpels per flower like seed-derived *L. peruvianum* (PrPrPr) plants. Grafts of the three chimeras onto stocks of *L. peruvianum* or tomato did not affect the phenotypes of the scions.

Determination of Floral Meristem Size

Scanning electron micrographs of meristems of *fasciated* tomato plants (fff), non-*fasciated* tomato plants (+++), and chimera +ff, shown in Figures 3 to 5, reveal that meristems of *fasciated* plants and the chimera are larger than the non-*fasciated* meristems at all stages of floral organ initiation. Scanning electron micrographs of chimera ++f revealed that meristem size at all stages of floral development was similar to those of *fasciated* plants and chimera +ff (data not shown). Diameters of freshly dissected vegetative and floral meristems, measured to the axils of the youngest organ primordia, as shown in Table 3, indicated that there was a progressive increase in meristem size during floral organ initiation of *fasciated* tomato plants (fff). Both chimera ++f and +ff showed a similar increase in size after the initiation of each successive whorl of organs. The meristem size during the initiation of each whorl of organs in these plants (fff, +ff, ++f) was significantly different from those of non-*fasciated* plants (+++). Sizes of

Table 2. Carpel Number in *L. peruvianum*, Tomato, and Chimeras[a]

	Carpels	SD	*n*
PrPrPr	2.0 x	0	20
EEE	5.2 y	0.83	20
PrEE	4.3 z	0.91	20
PrEPr	2.0 x	0	20
PrPrE	3.6 z	0.51	20

[a] Means for each organ type followed by a different letter (x, y, or z) differ significantly ($P < 0.0001$). A Kruskal-Wallis chi-square approximation test was used to test for differences between all pairs. Because multiple comparisons were made, the critical value was readjusted to reduce the overall probability of a type I error to <0.005 (Jones, 1984).

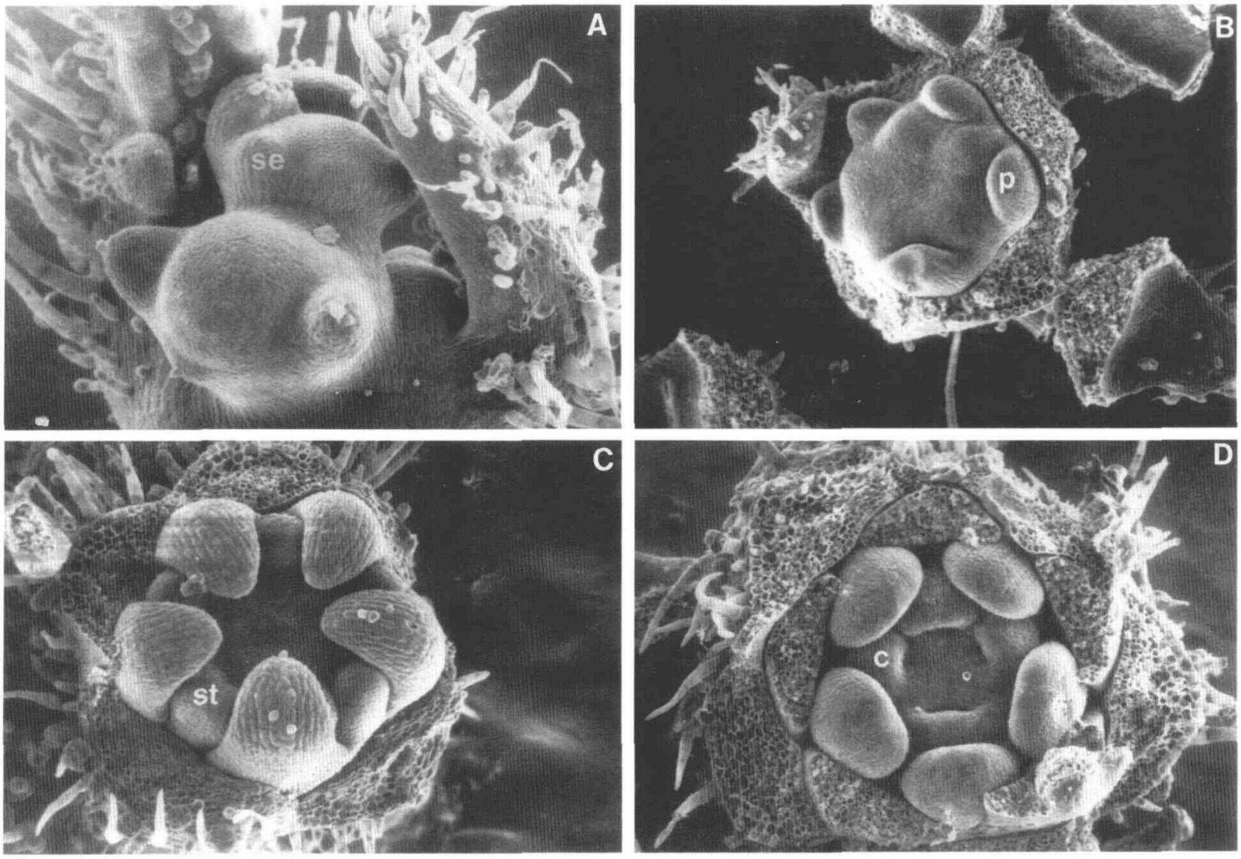

Figure 3. Floral Organ Initiation in Non-*fasciated* Tomato (+++).

(A) Sepals initiated (upper bud).

(B) Petals developing and stamens initiated (sepals removed).

(C) Petals and stamens developing, carpels initiated (sepals removed).

(D) Stamens and carpels developing (sepals and petals removed).

se, sepal; p, petal; st, stamen; c, carpel. Bar in **(A)** = 10 μm; all micrographs are at the same magnification.

vegetative meristems were not significantly different between *fasciated* tomato plants (fff) and non-*fasciated* tomato plants (+++) or the chimeras. Although stem fasciation as a result of increased meristem size during vegetative growth was observed in seed-grown plants prior to flowering, subsequent vegetative development from axillary buds did not exhibit an increase in meristem size. Because the chimeras needed to be vegetatively propagated from axillary buds, comparison to seed-grown plants was not possible.

Scanning electron micrographs of floral meristems early in carpel development of chimeras PrEE, PrPrE, and PrEPr, *L. peruvianum* (PrPrPr), and tomato (EEE), as shown in Figures 6A to 6E, reveal that PrEE, PrPrE, and EEE have meristems similar in size to and larger than those of PrEPr and PrPrPr at the time of carpel initiation.

DISCUSSION

Prior to organ initiation, a site on the meristem for a new primordium must be determined. Because primordia are multicellular in origin, information must be communicated to groups of cells at particular sites on the meristem to begin organ initiation, and information must be exchanged among the cells forming the primordium to coordinate their development. We have generated periclinal chimeras to determine if cells in particular positions in the meristem are the source of such information. In the chimeras described here, cells in the meristem may develop according to their own genotype or in response to information supplied to them from other cells in the meristem. Which of these two paths cells followed depended on their

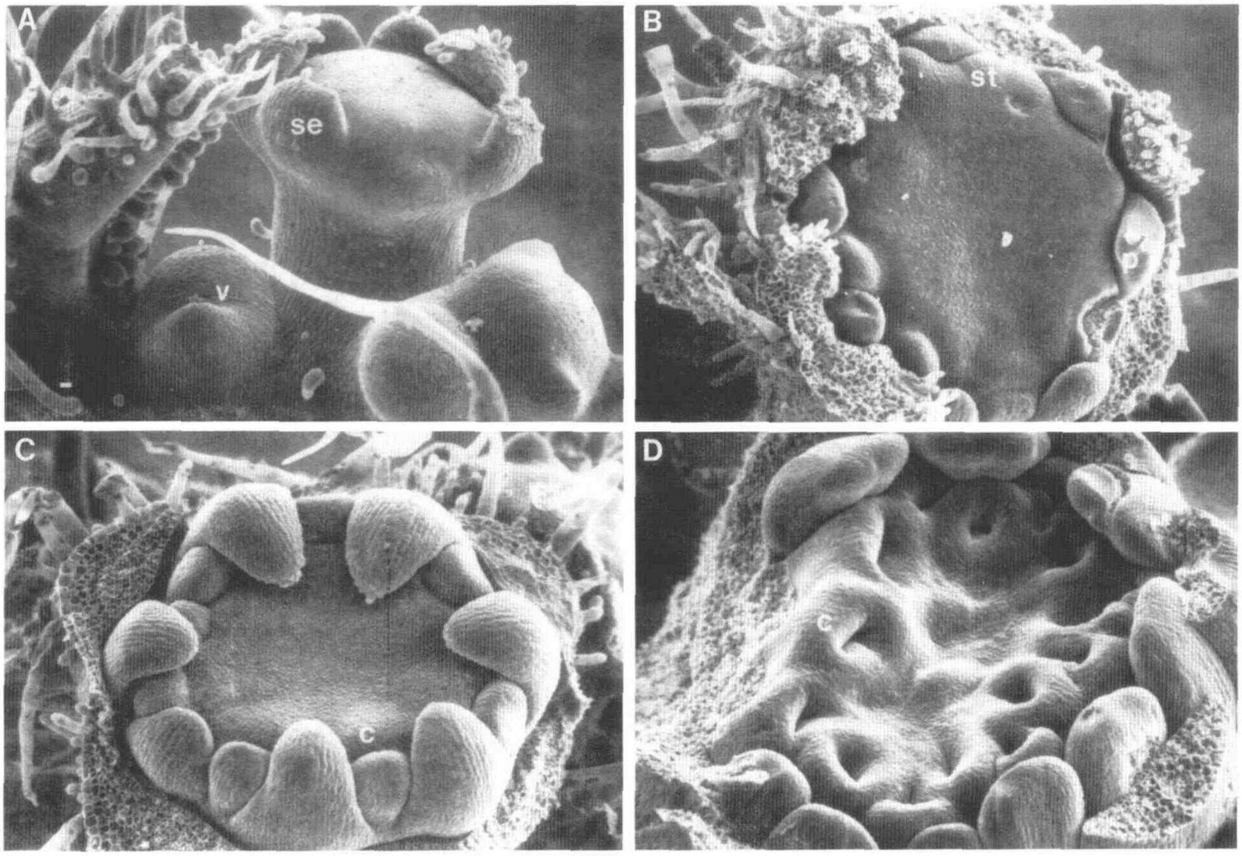

Figure 4. Floral Organ Initiation in Homozygous *fasciated* Tomato (fff).

(A) Sepals initiated (upper bud).
(B) Petals developing and stamens initiated (sepals removed).
(C) Petals and stamens developing, carpels initiated (sepals removed).
(D) Carpels developing.
v, vegetative meristem; se, sepal; st, stamen; p, petal; c, carpel. Bar in **(A)** = 10 μm; all micrographs are at the same magnification.

position in the chimeric meristems. Cells occupying L3 of the meristem determined meristem size during floral organ initiation and were the major determinant of carpel number. Cells in L1 and L2 contributed to floral meristem development predominantly in response to information supplied by cells in the L3 meristem layer.

For one set of chimeras, the mutation *fasciated* was used to increase carpel number. Although the molecular nature of this mutation is unknown, it provided a source of variation in the process of organ initiation. Chimeras +ff and ++f produced a number of floral organs in each whorl comparable to that of *fasciated* plants and many more than non-*fasciated* plants (Table 1). Chimera ++f initiated slightly fewer organs per whorl than *fasciated* plants. Although the genotype of cells in L3 had the greatest influence on organ number, the presence of wild-type cells in L2 caused a slight reduction in the number of organ primordia initiated. The non-*fasciated* L1 and L2 cells still participated in the initiation of many more floral organs than they would have if *fasciated* cells were not present in the meristem.

The increased number of floral organs observed in *fasciated* plants and chimeras +ff and ++f was correlated with an increased meristem size at the time of organ initiation (Table 3). The sizes of vegetative meristems in non-*fasciated* plants, *fasciated* plants, and chimeras +ff and ++f were all similar. The increase of floral meristem size in *fasciated* plants and the two chimeras resulted from more tissue being added to the meristem than was removed from the meristem by incorporation into organ primordia. From examination of scanning electron micrographs of +ff and ++f floral meristems, it is apparent that the wild-type L1 tissue that formed the surface layer of the large floral meristems had not undergone an increase in cell size, but an increase in cell number. This indicates that the internal *fasciated* cells induced the non-*fasciated* L1

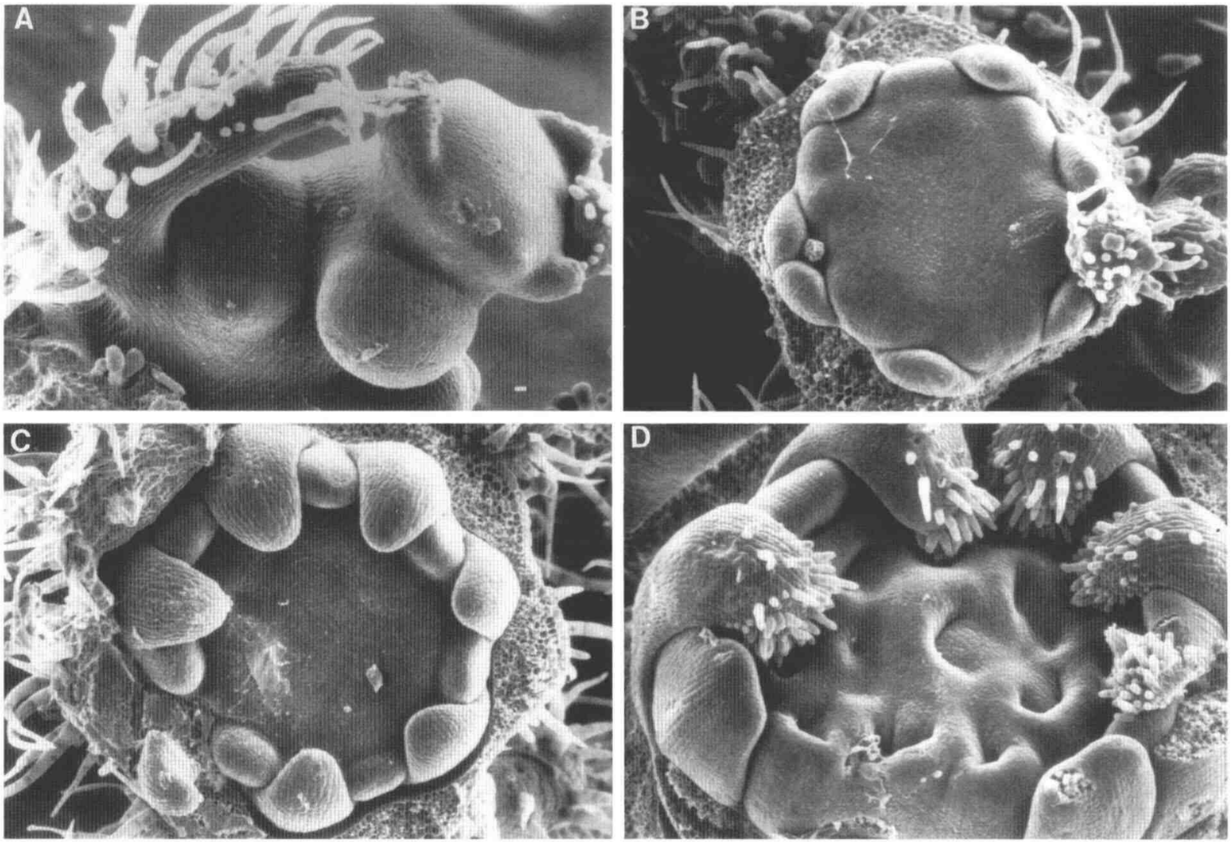

Figure 5. Floral Organ Initiation in Chimera +ff.

(A) Sepals initiated (upper bud).
(B) Petals developing and stamens initiated (all but one sepal removed).
(C) Petals and stamens developing, carpels initiated (sepals removed).
(D) Carpels developing.
Bar in **(A)** = 10 μm; all micrographs are at the same magnification.

cells to divide more frequently relative to the rate of organ initiation than occurs in non-*fasciated* plants. The same pattern of development occurred in L2 of chimera ++f, as indicated by histological examination of sectioned floral meristems (data not shown).

Chimeras +ff and ++f showed that the presence of a wild-type L1 or a wild-type L1 and L2 was not sufficient to cause the chimeric meristem to initiate wild-type numbers of floral organs. It cannot, however, be determined from these two chimeras if the genotype of L3 was critical in determining meristem size and primordium number or if *fasciated* cells merely needed to be present in the meristem to increase meristem size and primordium number. Chimeras with the remaining four cell layer combinations between *fasciated* and

Table 3. Diameters of Vegetative Meristems of Wild Type (+ + +), *fasciated* (fff), and Chimeras +ff and + +f[a]

	Vegetative	Sepal	Petal	Stamen	Carpel	*n*
+ + +	165.6 (20.0[b]) x	176.4 (8.1) x	187.2 (9.9) x	212.4 (8.1) x	212.4 (8.1) x	5
fff	172.8 (16.1) x	216.0 (25.5) y	259.2 (16.1) y	324.0 (25.5) y	417.6 (48.3) y	5
+ff	165.6 (19.7) x	208.8 (16.1) y	237.6 (19.7) y	288.0 (36.0) y	403.2 (30.1) y	5
+ +f	172.8 (16.1) x	201.6 (19.7) y	252.0 (25.5) y	295.2 (30.1) y	374.4 (32.2) y	5

[a] Means for each meristem stage followed by a different letter (x or y) differ significantly ($P < 0.0001$). Distances between means for each meristem stage were determined with the Student-Newman-Keuls test.
[b] SD.

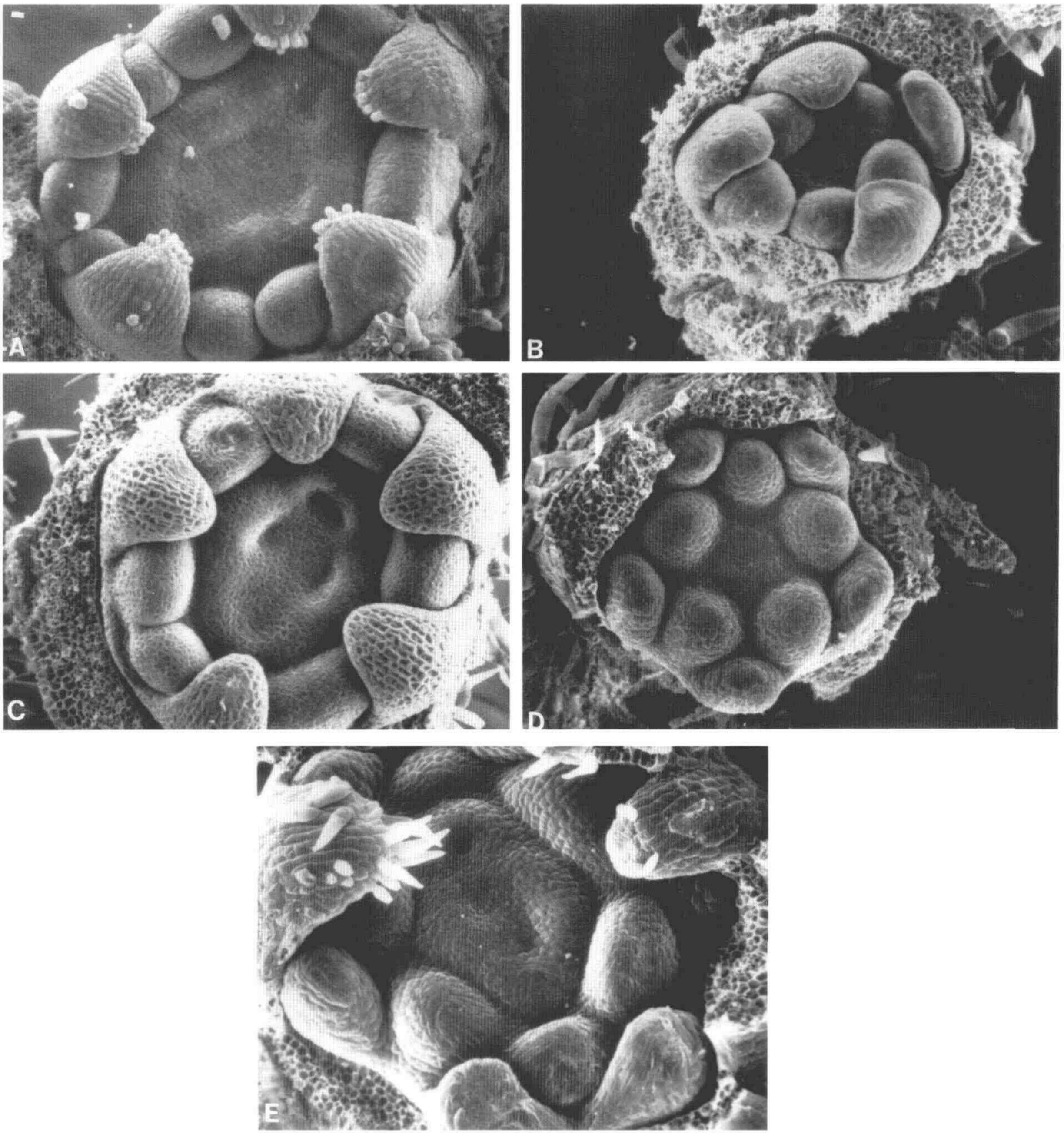

Figure 6. Carpel Initiation in Flowers of Tomato, *L. peruvianum*, and Three Chimeras.

(A) Tomato (EEE).
(B) *L. peruvianum* (PrPrPr).
(C) Chimera PrEE.
(D) Chimera PrEPr.
(E) Chimera PrPrE.
Bar in **(A)** = 10 μm; all micrographs are at the same magnification.

wild-type tomato were not obtained. However, the *L. peruvianum*–tomato chimeras were used to address this question.

In these chimeras, only the number of primordia initiated in the whorl of carpels differed significantly between *L. peruvianum* and the tomato line used. The number of carpels initiated by chimera PrEE was similar to that of tomato. The number of carpels initiated in chimera PrEPr was identical to that of *L. peruvianum.* Only the genotype of cells in L3 differed between these two chimeras. The number of carpels initiated in chimera PrPrE was greater than the number of carpels initiated by *L. peruvianum* (PrPrPr). The only difference between these two plants was the identity of cells in L3 of the meristem. This indicated that the genotype of cells located in L3 of the meristem was the primary determinant of the number of carpel primordia initiated, and as in the *fasciated* chimeras, this was correlated with an increase in meristem size at the time of carpel initiation. The presence of *L. peruvianum* cells in L1 and L2 had some effect on carpel number in chimera PrPrE, as compared to EEE; however, no influence on carpel number of tomato in L2 was observed in chimera PrEPr.

All of the chimeras described here indicated that L3 is important in defining floral meristem size and the number of floral organs initiated. Changes in cell layer identity as a result of atypical cell divisions in the meristem layers of some of the chimeras clearly demonstrated that the ability of L3 cells to coordinate floral organ initiation does not depend on their lineage but strictly on their position. For example, flowers on PrEE shoots that resulted from periclinal divisions in L2 of chimera PrEPr initiated a number of carpels similar to EEE and PrEE flowers and not like PrEPr flowers. This occurred even though the tomato cells in L3 of the PrEE shoots traced their lineage to L2 cells in chimera PrEPr.

The chimeras demonstrated that cells occupying L3 coordinated the incorporation of cells from all three meristem layers into organ primordia relative to the growth of the meristem. The mechanism by which L3 provides this information and the mechanism by which it is perceived by cells occupying L1 and L2 are unknown. The influence of L3 on L1 may be independent of the influence of L3 on L2. Alternatively, L2 may respond to L3, and in turn L1 responds to L2. Although *fasciated* affects the relation between growth of the meristem and organ initiation and L1 and L2 can respond to those effects in L3, the role of *fasciated* in cell interactions is not known. The action of *fasciated* may be limited to L3, and subsequent changes in L3 induce L1 and L2 to respond, or the *fasciated* gene product may directly signal L1 and L2 cells.

Theories of phyllotaxis attempt to explain the regular patterns of organ initiation during vegetative growth. One class of theories implicates fields of inhibition emanating from the apex summit and from previously initiated organs as defining future leaf initiation sites (Wardlaw, 1949). Similar processes have been implicated during floral organ initiation (Lyndon, 1978). These fields of inhibition could be in the form of a chemical gradient whose nature has not been determined, although auxin has been proposed (Schwabe, 1971). The chimeras described here suggest that if fields are acting to define primordium initiation sites, then cells of L3 are important in generating or interpreting such a field. Because sectored flowers have not yet been observed in which a fraction of L3 cells in the meristem is different from the rest of the meristem, it cannot be determined if the entire L3 is involved in this process or if only cells in certain regions of L3 are sufficient.

An alternative theory of phyllotaxis postulates that physical constraints generated by L1 cells of the meristem are responsible for the spacing and initiation of organ primordia (Green, 1985). During floral development, organ primordia are considered to be generated by localized bulging of the tunica, L1 and L2, as a result of alterations in the patterns of cellulose reinforcement on the meristem surface (Green, 1988). The chimeras described here show that during carpel initiation cells in L1 and L2 of the meristem did not define the pattern of organ initiation. If physical components of cells in L1 are critical in determining the pattern of primordium initiation, then it is apparent that these L1 cells must respond to signals that originate in the internal, L3 cells. This could proceed such that the internal cells determine the growth rate of the meristem, and L1 cells respond by generating the appropriate physical forces necessary for primordium outgrowth.

METHODS

Seed Stocks

Seed stocks of *Lycopersicon esculentum* lines carrying the mutations used here were provided courtesy of C. M. Rick, Department of Vegetable Crops, University of California, Davis; seed stock of *L. peruvianum* was obtained from A. E. Clarke, Plant Cell Biology Research Centre, University of Melbourne.

Cell Layer Markers

Several mutations that are autonomous in their phenotypic expression were used as markers to identify the genotype of cells in chimeric shoots. These markers are summarized in Table 4. Two mutations were used to mark the epidermal derivatives of L1. Plants homozygous for *Lanata* (*Ln*) have a greatly increased number of epidermal trichomes

Table 4. Mutations Used to Mark Cell Layer Derivatives

Mutation	Phenotype	Layer Marked
Lanata (*Ln*)	Increases trichome density	L1
hairless (*h*)	Lacks long trichomes	L1
Xanthophyllic-2 (*Xa-2*)	Yellow leaves, stem, and sepals	L2 and L3
anthocyanin gainer (*ag*)	Lacks anthocyanin in stem	L2

on the shoot, including stems, leaves, sepals, petals, and pistils. Plants homozygous for *hairless* (*h*) lack long epidermal trichomes on the shoot. Internal tissue (L2 and L3) was marked by *Xanthophyllic-2* (*Xa-2*). Plants heterozygous for *Xa-2* have yellow L2- and L3-derived leaf and sepal tissue and yellow L2-derived stem tissue. L2 was also marked by *anthocyanin gainer* (*ag*). Plants homozygous for *ag* lack anthocyanin in L2-derived stem tissue.

To generate the *fasciated* chimeras, two different lines of tomato were used. The marker line was *Xa-2/+*, *h/h*, *ag/ag*, and was wild type for *fasciated*. The *fasciated* line was homozygous for *fasciated* and was wild type for all cell layer markers. For the *L. peruvianum*–tomato chimeras, the tomato line carried *Xa-2/+* and *Ln/Ln*.

Generation of Chimeras

Chimeras were generated using a grafting technique similar to that described by others (Gunther, 1961; Clayberg, 1975; Marcotrigiano and Gouin, 1984). Seedlings were greenhouse grown in individual 4-inch-diameter pots until approximately three leaves had fully expanded. The three to four leaves above the oldest leaf were removed, and appropriate plants were reciprocally grafted at the second or third node above the cotyledons. The scion was fastened to the stock by wrapping several windings of stretched Parafilm M around the graft junction. Grafted plants were placed in an intermittent mist chamber for 7 to 10 days. The Parafilm was then removed, and the plants were returned to the greenhouse for 3 weeks. Cuts were then made through the graft junction, exposing cells from both the stock and scion at the cut surface. This surface was smeared with a thin film of petroleum jelly to help prevent desiccation. Callus usually formed at the cut graft junction, and shoots developed from the callus in as few as 10 days after cutting. If after 3 weeks no shoots developed, a second cut was made through the graft junction. As the shoots developed, they were screened for novel combinations of cell layer markers. Sectored shoots were allowed to grow until they reached a size that could be easily handled. They were then cut from the graft and rooted by placing them in soil under intermittent mist. Periclinal chimeras were obtained by selectively decapitating these sectored plants above a node containing a chimeric sector. This resulted in growth of the axillary bud at the node. Periclinal chimeric shoots were vegetatively propagated and grown to maturity. All plant material was grown in Metro Mix 2000 and fertilized biweekly with Rapid Grow fertilizer. Mature plants were grown in 10-inch-diameter pots in the greenhouse.

Grafts used to generate the *fasciated* chimeras and the *L. peruvianum*–tomato chimeras were continually made (greater than 100 for each set) and screened as greenhouse space allowed; grafting and screening continued for about 1 year.

Verification of Chimeras

The cell layer identity of the chimeras was determined primarily on the basis of the visible phenotypes of the cell layer autonomous mutations carried by one of the plants in each graft combination. These mutations are not expressed in the meristem itself; therefore, the identification of each layer was based on the phenotypes of tissues derived from the meristem. The mutations used to mark the internal layers were visible in leaves, stem, and sepals. The contribution of internal layers to mature carpels was visible in the mature fruit of chimeras between tomato and *L. peruvianum*, because *L. peruvianum* fruit do not turn red when ripe (Figure 1E), allowing identification of cells derived from all three meristem layers. Several techniques were used to verify further that the novel phenotypes were due to chimerism and not the result of spontaneous mutations. In other chimeras between plants of the Solanaceae, gametes were usually derived from cells in the L2 meristem layer (Tilney-Bassett, 1986). Therefore, the identity of L2 was confirmed by the phenotype of the progeny of self-pollinated chimeras. By decapitating young rooted cuttings of the chimeras, shoots that originated predominantly from genetically homogeneous groups of cells could be obtained from callus at the cut surface. In this way, pure shoots of the original genotypes used to generate the chimeras could be obtained from the chimeras. These shoots confirmed that the chimeras arise not by mutation but by the incorporation of genetically different cells into the layers of the apical meristem.

Tissue Culture

Additional chimeras with different cell layer combinations were obtained from the original chimeras by regenerating shoots in tissue culture. Expanding leaves were sterilized by soaking in 15% Clorox for 15 min, then were rinsed in two changes of sterile distilled water. Discs were cut from the leaves and placed in Petri dishes containing regeneration medium (Tatchell and Binns, 1986), consisting of MS salt base (JRH Biosciences, Lenexa, KS), 1 mg/L thiamine HCl, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine, 3 μM indoleacetic acid–aspartic acid, 5 μM zeatin riboside, 30 g/L sucrose, 9.5 g/L agar. The medium was sterilized after the hormones were added. Plates were placed in a plant growth chamber that provided 16 hr of light per day. Shoots obtained from the cultured leaf discs were transferred directly to the mist chamber and rooted in soil where they were screened for chimeric sectors.

Electron Microscopy

Specimens were fixed in ice cold FAA (5% formalin, 5% acetic acid, 50% ethanol) for a minimum of 8 hr. The samples were then chemically dehydrated in two changes of 2,2-dimethoxypropane (Sigma) that was acidified with 0.05 mL of 0.1 M HCl per 25 mL (Lin et al., 1977). Samples were then transferred to 100% ethanol and dried in a critical point dryer. Dried specimens were sputter coated with paladium and gold and observed in a ISI SS-40 scanning electron microscope.

Determination of Floral Organ Number

Floral organ number was determined from flowers at anthesis. For comparison with the *fasciated* chimeras, +++ and fff flowers were obtained from cuttings of both the self-pollinated progeny of the chimeras and cuttings of the original plants used to generate the chimeras. EEE and PrPrPr carpel numbers were determined from cuttings of the original plants used to generate the chimeras.

Determination of Meristem Size

Freshly dissected meristems of various stages were examined under a Wild M5 stereomicroscope. Diameters were measured between the axils of the youngest primordia by comparison to an ocular micrometer. Vegetative meristems were measured from growing axillary buds.

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